REMARKS

Attached is a marked-up version of the changes made to the specification by the current amendment. The attached page is captioned "**Version with markings to show changes made.**"

Respectfully submitted,

Date: 7/13/01 By: _____

Roberta L. Robins
Registration No. 33,208

ROBINS & PASTERNAK LLP
90 Middlefield Road, Suite 200
Menlo Park, CA 94025
Telephone: 650-325-7812
Facsimile: 650-325-7823

VERSION WITH MARKINGS TO SHOW CHANGES MADE

Paragraph beginning at page 8, line 4, has been amended as follows:

Figure 7 depicts a multiple sequence alignment of DWF7/STE1 with known sequences for Δ^7 sterol C-5 desaturases. The GenBank accession numbers for the sequences are M62623 (*S. cerevisiae*) (SEQ ID NO:16 [__]), AB004539 (*Schizosaccharomyces pombe*) (SEQ ID NO:17 [__]), L40390 (*C. glabrata*) (SEQ ID NO:15 [__]), and AF105034 (DWF7/STE1, Arabidopsis) (SEQ ID NO:18 [__]). The conserved transmembrane domains and histidine clusters are boxed and labeled. The positions of the premature stop codons in *dwf7-1* and *dwf7-2* are indicated with filled circles. Histidine residues in each conserved histidine box are identified with filled triangles. A consensus sequence (SEQ ID NO:19 [__]) is shown in the bottom row of the alignment. Capital letters stand for residues conserved among all sequences, whereas lowercase letters mean ≥50% identical. Dashes indicate gaps introduced to maximize alignment. Multiple sequence alignment was performed using PILEUP in the Genetics Computer Group software (Madison, WI) with a gap creation penalty of 4 and a gap extension parameter of 1. The annotation of the aligned sequences was performed using the ALSCRIPT software (Barton (1993) Protein Eng. 6:37-40).

Paragraph beginning at page 8, line 19, has been amended as follows:

Figures 8A-8D (SEQ ID NO:20) depict the complete gene sequence of *dwf7*, denoted by a dark grey bar. The premature stop codons for dwf7-1 and dwf7-2 are shown with triangles at nucleotide positions 1552 and 322, respectively. The coding sequence and corresponding amino acid sequence are represented by a light grey bar. The mRNA

sequence is represented by a black bar and is shown in three segments. The gene includes two introns (positions 369-735 and 1042-1395) and three exons.

Paragraph beginning at page 8, line 25, has been amended as follows:

Figure 9 (SEQ ID NO:21) shows the amino acid sequence corresponding to the coding sequence designated in Figures 8A-8D. The polypeptide sequences corresponding to the *dwf7-2* and *dwf7-1* alleles occur at positions 1-60 (SEQ ID NO:24) and 1-230 (SEQ ID NO:25), respectively.

Paragraph beginning at page 8, line 28, has been amended as follows:

Figures 10A-10F (SEQ ID NO:22) show the gene sequence of the *dwf7* homologue, *HDF7*. The coding sequence and corresponding amino acid sequence are shown in three segments (exons), occurring at positions 1506-1734, 2024-2329 and 2416-2720 of the figure. The 5' UTR is shown at positions 1-1505 and the 3' UTR occurs at positions 2721-2925.

Paragraph beginning at page 9, line 3, has been amended as follows:

Figure 11 (SEQ ID NO:23) shows the amino acid sequence corresponding to the coding sequence designated in Figures 10A-10F. The polypeptide sequence corresponding to the HDF7 dwf7 polypeptide occurs at positions 1-230 of the figure.

Paragraph beginning at page 40, line 24, has been amended as follows:

PCR products amplified using primer sets derived from the cDNA sequence of *STEROL1* (*STE1*) were subjected to sequencing. To design sets of primers that do not

fall in exon-intron junctions, we predicted possible splice sites by using the RNASPL program available at the internet site of Baylor College of Medicine (Houston, TX; http://dot.imgen.bcm.tmc.edu:9331/seq-search/gene-search.html). Primers were designed using the Primer Selection software of DNAstar (DNASTAR Inc., Madison, WI). Oligonucleotide sequences 5' to 3' are CAGTGTGAGTAAT T TAGCAT TACTA (S5D_FF) (SEQ ID NO:1), GGAAAGATCATC-AAACAT T TACATGT (S5D_LR) (SEQ ID NO:2), GCGCAATCT TCT T TCGT T T (S5D_1F) (SEQ ID NO:3), TGGACAACAACAACACAAGA (S5D_1R) (SEQ ID NO:4), GATGCACAGAGAGCT- TCATGAC (S5D_2F) (SEQ ID NO:5), CCGGCAAATGGAGAGAGTGTAT (S5D_2R) (SEQ ID NO:6), CACCCATCATATCTACAACAA (S5D_3F) (SEQ ID NO:7), and CATCT T T TGCCG-GCGAATCTAT (S5D_4F) (SEQ ID NO:8) (underlines were added to distinguish forward or reverse primers from the gene acronym S5D). Primers were purchased from Genosys Biotechnologies, Inc. (The Woodlands, TX). For template DNA, genomic DNA was isolated from two or three leaves of *dwf7-1* and wild-type plants according to the method described by Krysan et al. (1996) Proc. Natl. Acad. Sci. USA 93:8145-8150. Amplification of the DNA fragment spanning the whole coding region was performed with the S5D_4F and S5D_1R primer set with Taq polymerase (Boehringer Mannheim).

Paragraph beginning at page 42, line 3, has been amended as follows:

Genomic DNA sequence flanking the cDNA was identified by sequencing the products obtained from thermal asymmetric interlaced PCR (TAIL PCR) (Liu et al. (1995) Plant J. 8:457-463). Two sets of primers were used to amplify the 5' and 3' flanking DNA. Oligonucleotide sequences 5' to 3' are GTAGAAGCACCAGAGGAAACCGGAGATGAAGT (D7-5-1; melting temperature of 69°C) (SEQ ID NO:9), AAGTATAGTAGGGT TCCGGCGAGG-TA (D7-5-2; melting

temperature of 64°C) (SEQ ID NO:10), ATAGAT TCGCCG-GCAAAAGATGACTC (D7-5-3; melting temperature of 63°C) (SEQ ID NO:11), TGC-AGGATACCATACGATACACCACACGACAT (D7-3-1; melting temperature of 68°C) (SEQ ID NO:12), CATACGATACACCACACGACATACAAGCAT-AACTA (D7-3-2; melting temperature of 67°C) (SEQ ID NO:13), and ATATGGATG-GAT TGGATGT T TGGCTCTC (D7-3-3; melting temperature of 63°C) (SEQ ID NO:14). The melting temperature of each primer was calculated with the formula 69.3 + 0.41 (%GC) - 650/L (Mazars et al. (1991) Nucleic Acids Res. 19:4783), where L is length of primer. Arbitrary degenerate primers AD1, AD2, and AD3 were synthesized according to the sequence described by Liu et al. (1995) Plant J. 8:457-463. TAIL PCR was performed according to the program originally described by Liu et al. 1995. TAIL PCR-amplified DNA was separated on 1% agarose gels and gel extracted for sequencing.